Abnormal promoter DNA methylation in juvenile myelomonocytic leukemia is not caused by mutation in DNMT3A

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DOI: https://doi.org/10.1182/blood-2011-07-370684

To the editor:

Abnormal promoter DNA methylation in juvenile myelomonocytic leukemia is not caused by mutation in *DNMT3A*

Several recent publications reported the occurrence of mutations in the DNA methyltransferase 3A (*DNMT3A*) gene in acute myeloid leukemia (AML).[1-3] The majority of *DNMT3A* mutations identified affect residue R882 which is located within the methyltransferase domain.[1,2] A strong predilection for leukemias of monocytic or myelomonocytic lineage was observed.[2,3] This, and our previous findings linking altered DNA methylation patterns with poor prognosis in juvenile myelomonocytic leukemia (JMML),[4] led us to hypothesize that somatic *DNMT3A* mutations might also occur in JMML.

We bidirectionally sequenced *DNMT3A* exon 23 (containing the hotspot codon 882) in granulocyte DNA from 113 JMML patients. All children were enrolled in the European Working Group of MDS in Childhood (EWOG-MDS) studies 98 or 2006, and informed consent had been obtained from patients' guardians. The patients' median age was 2.0 years. The hotspot mutation *DNMT3A* p.R882H was identified in JMML patient D101 (age at diagnosis 6.5 years, somatic *NRAS*^{G12V} mutation; Figure 1A), while all other samples exhibited wild-type sequence. Epstein-Barr virus-transformed B lymphocytes of D101 carried wild-type sequence, indicating somatic origin of the *DNMT3A* mutation.

Although *DNMT3A* is generally overexpressed in AML blasts versus healthy leukocytes,[5] the expression does not correlate with occurrence of *DNMT3A* mutations.[1] Despite the finding that *DNMT3A* codon 882 mutations lead to reduced enzymatic activity,[2] the genome-wide DNA methylation pattern of *DNMT3A* codon 882-mutant AML cells does not differ significantly from that of *DNMT3A* wild-type blasts.[1] We measured *DNMT3A* mRNA expression in mononuclear cells from 9 JMML patients by quantitative real-time PCR (RNA from patient D101 was unavailable). The abundance of the *DNMT3A* transcript in JMML cells varied considerably, with some cases expressing *DNMT3A* at higher levels than leukocytes from healthy subjects, and others at lower levels (range, 0.08-14.05 fold; Figure 1B). Unlike in AML where uniform blast populations are studied, our results may reflect the heterogeneous cellular composition of JMML samples. DNA methylation data were available for 6 of the 9 JMML samples studied,[4] but there was no association of high *DNMT3A* expression with DNA hypermethylation (Figure 1C). Remarkably, leukemic cells of D101 carried hypermethylated *BMP4*, *CALCA* and *CDKN2B* promoters[4] despite the presence of a *DNMT3A* loss-of-function mutation.

Several investigators highlighted the possible pathophysiologic role of the DNA-methylating enzyme DNMT3A in myeloid malignant disorders other than JMML.[1-3,6,7] We conclude that genetic or transcriptional aberrations of *DNMT3A* do not contribute to leukemogenesis in JMML. While the recurrent nature of *DNMT3A* alterations in AML, the strong preference to target codon 882 and the prognostic significance argue in favor of non-random pathogenetic relevance in AML, a DNA methylation profile that would distinguish *DNMT3A*-mutant AML cases has not yet been defined. By contrast, JMML cases with poor outcome exhibit promoter hypermethylation of several genes[4] but for the most part lack *DNMT3A* alterations. The data presented here fit well with previous observations that *DNMT3A* mutations are much less prevalent in myeloid malignancies of children than in adults.[6,8] In summary, the functional implication of *DNMT3A* lesions in myeloid leukemogenesis requires further clarification.

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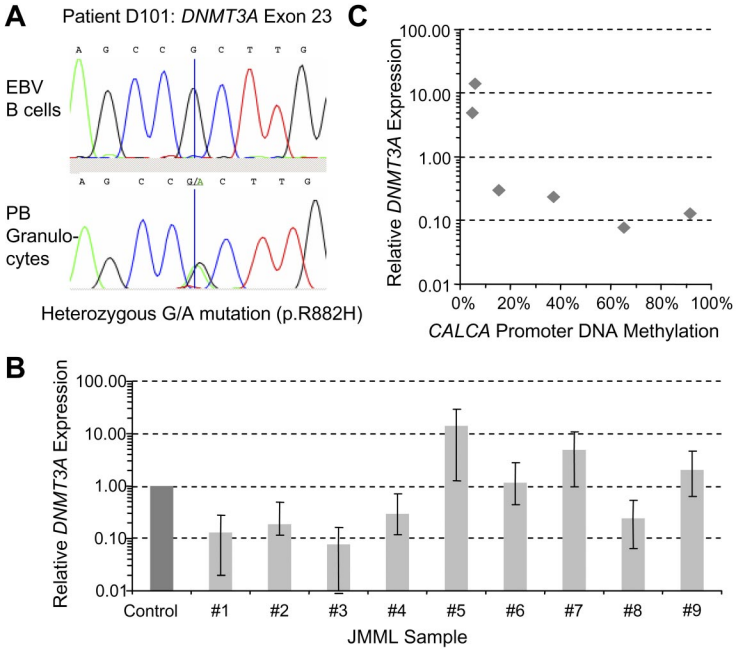

Figure 1. DNMT3A mutation p.R882H in JMML patient D101, *DNMT3A* mRNA expression in JMML mononuclear cells and correlation with promoter DNA methylation. (A) Heterozygous *DNMT3A* R882H mutation detected in granulocytes but not in Epstein-Barr virus (EBV)–transformed B cells of patient D101. PB indicates peripheral blood. (B) Expression of *DNMT3A* mRNA was measured by quantitative real-time PCR in mononuclear cells from 9 patients with JMML. Expression in normal controls (n = 4) was averaged and set to 1.0. Each sample was measured in triplicates; error bars denote 1 SD. (C) Expression of *DNMT3A* mRNA and calcitonin A (*CALCA*) gene promoter DNA methylation in 6 cases of JMML (methylation data previously published in Olk-Batz et al[4]).

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Acknowledgments: The authors thank Barbara De Moerloose (Ghent, Belgium), Jan Starý (Prague, Czech Republic), Marco Zecca (Pavia, Italy), Franco Locatelli (Rome, Italy), Dorota Wojcik (Wroclaw, Poland), Markus Schmugge (Zurich, Switzerland), Marry M. van den Heuvel-Eibrink (Rotterdam, The Netherlands) and all collaborators of the European Working Group of Myelodysplastic Syndromes in Childhood for contributing clinical data and research material.

This work was supported by the Deutsche Forschungsgemeinschaft grant (KR3473/1-1 to C.F.).

Conflict-of-Interest disclosure: The authors declare no competing financial interests.

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To the editor:

Is there still a place for myeloablative regimen to transplant young adults with sickle cell disease?

We have read with great interest the comprehensive and detailed review on hematopoietic stem cell transplantation (HSCT) for sickle cell disease (SCD) published by Hsieh et al in a recent issue of the journal.[1]

As emphasized by the authors, allogeneic HSCT is currently the only available curative treatment for SCD, but is clearly underutilized. Although it is now considered by many teams as the "standard of care" for symptomatic children with an HLA identical sibling, the experience in adults remains very limited.

In their paper, the authors claim that the development of a SCD-specific transplant regimen based on nonmyeloablative conditioning, along with the use of peripheral blood donor cells and long-term immunosuppression will provide stable mixed chimerism without rejection or GVHD.[2] This innovative approach is proposed with the implicit rationale that classic transplantation regimens used for hematologic malignancies are too toxic and dangerous for SCD adult patients who have accumulated organ failures and comorbidities before transplant.

We believe that classic myeloablative HSCT regimen has still a place for young adults with severe SCD, and in this context, we wish to report the French experience in 15 patients older than 16 years of age (Table 1) who have received geno-identical HSCT after a full-dose conditioning regimen, 4 of them having been reported previously.[3]

All patients received the same BU-CY-ATG regimen[3] and GVHD prophylaxis with short-methotrexate and cyclosporine. Only 1 death was observed in a patient with severe cerebral vasculopathy and Moya-Moya who suffered of massive cerebral hemorrhage at day 32 posttranplant despite a successful engraftment. Seven patients experienced grade 2 and one grade 3 acute-GVHD, quickly resolved with prednisone. Other complications included seizures, pericarditis, hemorrhagic cystitis, a successfully evacuated sub-dural hematoma and an episode of prolonged but ultimately resolved thrombopenia. Only 2 patients experienced moderate chronic-GVHD. With a median follow-up of 3.4 years (range = 1-16.1), overall disease-free survival was 93.3% $\pm$ 0.12. All survivors currently enjoy a normal quality of life without immunosuppression. Chimerism at 1 year was full-donor in 12 patients and mixed but $>$ 75% donor in 2 patients.

Although limited, this experience is the largest so far, and demonstrates that full myeloablative transplantation has an acceptable toxicity in selected young adults with SCD. In fact, we did not observe any unusual complications because of accumulated organ failures such as veno-occlusive disease or renal failure. The post-HSCT period had more complex events than in younger patients but remained manageable. As opposed to nonmyeloablative transplantation, full-dose regimen carries much less risk of rejection, does not necessitate prolonged immunosuppressive